

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: KLINEFELTER 1C

In re Application of:) Art Unit: 1644
Gary KLINEFELTER) Examiner: P.J. NOLAN
Serial No.: 09/752,514) Confirmation No. 9981
Filed: January 3, 2001) Washington D.C.
For: METHOD FOR EVALUATING)
AND AFFECTING MALE)
FERTILITY)

DECLARATION

I, Gary R. Klinefelter, do hereby declare that I am the sole inventor of the above-captioned application.

Attached hereto are photomicrographs of plates generated using SP22 antibody as described in the subject application and in the copy of Klinefelter et al., *Journal of Andrology* 23(1): 2002, as well as graphs which compare the amino acid sequences recognized by SP22 and DJ-1 antibodies.

In the graph submitted herewith, the assay consists of 15 mer peptides, each overlapping by 12 amino acids. The entire 189 amino acid sequence of SP22 is represented on this graph. Thus, the immunoreactive amino acid sequences (peaks) represent the linear epitopes for each antibody. There are four distinct epitopic peaks (amino acid sequences) recognized by the SP22 antibody and

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one amino acid sequence recognized by the DJ-1 antibody. Since the epitope recognized by the DJ-1 antibody is one of the epitopes recognized by the SP22 antibody, and only the SP22 antibody recognizes the sperm surface, it is clear that not all antibodies that recognize DJ-1 will recognize SP22, the sperm surface molecule.

The plates shown in the photomicrographs attached hereto were generated using the SP22 antibody as described in the attached reprint of Klinefelter et al. (*ibid*). The top two micrographs depict immunostaining of fresh unfixed rat sperm visualized by epifluorescence. There is no fluorescent staining on sperm when the DJ-1 antibody is used. In contrast thereto, the equatorial region of the sperm head (indicated by an arrowhead) is immunostained specifically using antibody to SP22.

The bottom four micrographs depict immunostaining of fresh unfixed human sperm. The micrographs on the left of the sheet depict sperm visualized by phase illumination only. The micrographs on the right show the same image viewed by epifluorescence. The sperm show no specific immunostaining with DJ-1 antibody. In contrast thereto, antibody to SP22 stains the equatorial segment of the sperm head (see arrowhead) as well as the cytoplasmic droplet.

Figures 3 and 9 in the Klinefelter et al. article also

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demonstrate this specific staining over the equatorial segment of the sperm head.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon:


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